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(54) Title: METHOD FOR TREATMENT OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS) USING TRIPTOLIDE COMPOUNDS

(57) Abstract: The use of triptolide compounds for treatment of SARS infection is disclosed. The compounds are effective to inhibit cytokine production and thereby reduce symptoms, particularly in the immune hyperactive phase of the disease.

Method for Treatment of Severe Acute Respiratory Syndrome (SARS) using Triptolide Compounds

Field of the Invention

The invention is directed to treatment of SARS infection, and in particular to use of triptolide compounds to inhibit cytokine production in SARS patients.

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Background of the Invention

Severe Acute Respiratory Syndrome (SARS) is a serious public health threat. Although identification and isolation of infected individuals contributes to limitation of the spread of SARS infection, periodic renewed outbreaks and reemergence of the infection in patients previously thought to have recovered from SARS indicate the ongoing unmet need for improved therapy for SARS.

SARS is caused by a coronavirus (the SARS coronavirus, or SARS-CoV) and is a serious, contagious, rapidly progressing disease. The life-threatening aspect of SARS involves the lungs, and appears to result from a progression of pulmonary disease involving diffuse alveolar damage, a fibrosis-like condition in the lungs, and acute respiratory distress. There is evidence that the viral content in the lungs of SARS patients peaks and begins decreasing before the clinical severity of SARS symptoms reaches a peak. Based on the experience in Hong Kong, the SARS clinical course has been divided into three phases: the viral replicative phase, the immune hyperactive phase, and the lung destructive phase.

Changes in lung tissue indicate that cytokine production is involved in the pathologic effects upon the lungs (Drosten *et al.*, 2003). In acute viral respiratory infections, early-response cytokines such as IFN-γ, TNF-α, IL-1β and IL-6 mediate lung injury (Lee *et al.*, 2003). Respiratory viruses, particularly certain types of influenza virus, are potent inducers of proinflammatory cytokines and produce cytokine disregulation, which contributes to the pathogenesis and unusual severity of some human influenza viral infections (Cheung *et al.*, 2002). Patients with the highly virulent 1997 avian flu had primary viral pneumonia complicated by acute respiratory distress, a syndrome that is associated with cytokine disregulation (Headley *et al.*, 1997).

The reduction of virus (SARS-CoV) content before the peak of the disease process and the usefulness of corticosteroid treatment, as noted below, suggest that the development of the most severe, life-threatening effects of SARS may result from the

exaggerated response of the body to the infection (immune hyperactivity) rather than effects of the virus itself. Corticosteroid treatment is used in SARS patients to suppress the massive release of cytokines that may characterize the immune hyperactive phase, in the hope that it will stop the progression of pulmonary disease in the next phase.

Corticosteroid treatment has produced good clinical results. In many cases, lung shadows on X-rays start to resolve, and oxygenation improves after corticosteroid treatment. The majority of SARS patients receiving corticosteroid therapy in one clinic responded with resolution of fever and lung opacities within two weeks. Hydrocortisone or prednisolone has become first-line therapy, and a 2-day i.v. course of methylprednisolone is used in the most severe cases in most patients.

Although corticosteroids appear to reduce some of the major symptoms of SARS, there are several treatment-related side effects, and there is a clear need for more selective agents.

Summary of the Invention

The invention provides, in one aspect, a method of inhibiting cytokine production in a patient infected with Severe Acute Respiratory Syndrome (SARS) coronavirus, thereby reducing symptoms of the disease, comprising administering to such a patient a triptolide compound. The triptolide compound may be triptolide, a prodrug of triptolide, a derivative of triptolide which has cytokine inhibiting ability, or a prodrug thereof. Such compounds are described further below. For example, an exemplary triptolide prodrug is a pharmaceutically acceptable triptolide 14-succinate salt, such as a sodium salt.

In a related aspect, the invention is directed to the use of a triptolide compound, selected from triptolide, a triptolide prodrug, and a triptolide derivative or prodrug thereof having cytokine inhibiting ability, for preparation of a medicament for inhibiting cytokine production in a patient infected with Severe Acute Respiratory Syndrome (SARS) coronavirus.

Administration of the triptolide compound is preferably initiated when the level of SARS-CoV in the lungs of the patient has declined from a peak level, indicating that the patient has reached the second stage (immune hyperactive stage) in the clinical course of the disease.

The triptolide compound may be employed in combination with an additional therapeutic agent selected from an antiviral agent, an antiinflammatory agent, such as a

corticosteroid, an additional immunosuppressive agent, and an immune potentiator. Such agents are also described further below.

Detailed Description of the Invention

I. Triptolide Compounds

In accordance with the present invention, triptolide, triptolide derivatives and triptolide prodrugs (referred to collectively as triptolide compounds) are effective to inhibit cytokine production and are thus useful in the treatment of SARS, alone or in combination with other immunosuppressive agents.

The compound triptolide, a diterpene triepoxide isolated from the Chinese medicinal plant *Tripterygium wilfordii*, has potent immunosuppressive and antiinflammatory properties and reduces T lymphocyte proliferation and recruitment (Qui *et al.*, 1999). The compound suppresses *in vitro* production of proinflammatory cytokines such as IFN-γ, TNF-α, IL-1β and IL-6, as shown in Table 1.

To obtain the data, Jurkat cells were stimulated for 8 hr by PMA and ionomycin. Human peripheral blood mononuclear cells (PBMC) from a single donor were incubated for 24 hr with PHA. At the end of the culture period, each supernatant was harvested, and the cytokine content was assayed by ELISA.

Table 1. Suppression of cytokine production by triptolide

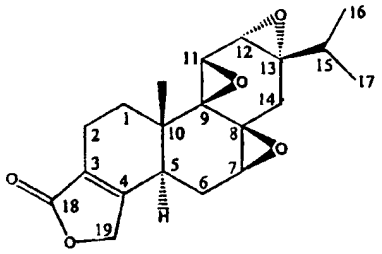
Cells and stimulus	Cytokine	IC_{50} (ng/ml)
PMA/ionomycin-induced Jurkat cells	IL-2	1.3
PHA-induced PBMC	IL-1β	0.45
	IL-2	0.38
	IL-6	1.5
	TNF-α	0.35
	IFN-γ	0.52

Triptolide suppresses the production of cytokines in a variety of *in vitro* systems. For example, triptolide inhibits early cytokine gene expression in Jurkat T cells, effectively suppressing T lymphocyte activation (Qui *et al.*, 1999). Triptolide inhibits production of IL-2 in activated human peripheral blood mononuclear cells (PBMC) and in activated Jurkat cells (Table 1; see Qui *et al.*, 1999, 2003). The secretion of the proinflammatory cytokines IFN-γ, TNF-α, IL-1β and IL-6 by PHA-activated human PBMC is also

suppressed by triptolide (Table 1). Triptolide inhibits the expression of several cytokine genes in activated Jurkat cells, including IL-2, IL-3, IL-6, IL-8, IL-13, TNF-α, TGF-β, MIP-1α, MIP-1β, GM-CSF and RANTES (Qui *et al.*, 2003). In addition to its effects on immune cells, triptolide suppresses IL-8 expression by bronchial epithelial cells, inhibiting both IL-8 mRNA and IL-8 protein expression (Qui *et al.*, 1999).

Triptolide derivatives and prodrugs which can be used in the method of the invention include those described in several co-owned US patents, including U.S. Patent Nos. 5,663,335, 6,150,539, 6,458,537, 5,962,516, and 6,569,893, and in co-owned PCT Pubn. No. WO 2003/101951, each of which is hereby incorporated by reference in its entirety. These derivatives and prodrugs can be prepared from triptolide, as described therein.

For the purposes of the current disclosure, the following numbering scheme is used for triptolide compounds:



An exemplary triptolide prodrug, triptolide 14-succinate (designated PG490-88; see U.S. Patent No. 5,663,335), is converted *in vivo* to triptolide by the action of esterases in plasma. The compound has shown *in vitro* activity in suppression of IL-2 production after incubation in plasma, and has shown efficacy in several animal models of immunosuppression (see *e.g.* Chen *et al.*, 2000; Wang *et al.*, 2000; Chen *et al.*, 2002; Fidler *et al.*, 2002).

Further exemplary triptolide derivatives and prodrugs which can be used in the method of the invention include 14-methyltriptolide (designated PG670; see US application serial no. 10/738,753), triptolide 14-tert-butyl carbonate (designated PG695; see US application serial no. 10/478,777 and corresponding PCT Pubn. No. WO 03/101951), 14-deoxy-14α-fluoro triptolide (designated PG763; see US application serial no. 10/786,663), triptolide 14-(α-dimethylamino)acetate (designated PG702; see U.S. Patent No. 5,663,335), 5-α-hydroxy triptolide (designated PG701; see U.S. application serial no. 60/532,702), 14-acetyl-5,6-didehydro triptolide (designated PG746; see U.S. application serial no. 60/532,702), 19-methyl triptolide (designated PG795; see U.S.

application serial no. 60/549,769), and 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (designated PG796; see U.S. application serial no. 60/549,769). Each of these compounds has demonstrated cytokine inhibiting activity, as shown in the above-referenced patents and applications. For example, PG796 (18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide) showed a higher level of activity in a standard IL-2 inhibition assay than the known triptolide prodrug, triptolide 14-succinate (PG490-88). Both 5α-hydroxy triptolide (designated PG701) and 14-acetyl-5,6-didehydro triptolide (designated PG746) inhibited IL-2 production in Jurkat cells in a dose-dependent manner at concentrations of about 10 nM or greater (the latter after incubation for 16 hours with human serum, which presumably removes the acetyl group). The activity of PG763 (14-deoxy-14α-fluoro triptolide) in assays evaluating cytotoxicity and IL-2 inhibition was nearly equivalent to that of native triptolide.

Methods of preparation of these and related compounds are described in the above-referenced patents and applications, and several exemplary procedures are reproduced below in the Examples. Each of these U.S. applications and patents is hereby incorporated by reference in its entirety.

Any of the above triptolide compounds having an ionizable group at physiological pH may be provided as a pharmaceutically acceptable salt. This term encompasses, for example, carboxylate salts having organic and inorganic cations, such as alkali and alkaline earth metal cations (for example, lithium, sodium, potassium, magnesium, barium and calcium); ammonium; or organic cations, for example, dibenzylammonium, benzylammonium, 2-hydroxyethylammonium, bis(2-hydroxyethyl) ammonium, phenylethylbenzylammonium, dibenzylethylenediammonium, and the like. Other suitable cations include the protonated forms of basic amino acids such as glycine, ornithine, histidine, phenylglycine, lysine, and arginine.

Many of these triptolide compounds act as prodrugs, by converting *in vivo* to triptolide, as observed for PG490-88, above. Compounds which are expected to convert to triptolide *in vivo* by known mechanisms (such as hydrolysis of an ester (organic or inorganic), carbonate or carbamate to an alcohol, or ring opening or ring closure from or to an epoxide or lactone) are referred to herein as prodrugs of triptolide. Such compounds are typically designed with such conversion in mind. These include, of those noted above, triptolide 14-succinate, triptolide 14-t-butyl carbonate and triptolide 14-(α-dimethylamino) acetate. Other prodrugs are described in U.S. Patent Nos. 5,663,335, 5,962,516,

6,150,539, 6,458,537, and 6,569,893, and PCT Pubn. No. WO 2003/101951.

Other triptolide compounds, such as 14-deoxy-14α-fluoro triptolide, 14-methyl triptolide, 5-α-hydroxy triptolide, 14-acetyl-5,6-didehydro triptolide, 19-methyl triptolide, and 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide, noted above, are not expected to undergo conversion to triptolide by a predictable mechanism, but nonetheless exhibit biological activities shown by triptolide (*e.g.* cytotoxicity in human T cell lymphoma (Jurkat) cells and immunosuppressive activity, such as inhibition of IL-2), as reported, for example, in the US applications and patents cited above. Compounds in this category are referred to herein as non-prodrug derivatives, or simply derivatives, of triptolide. (Note that while such compounds could in fact be converted to triptolide *in vivo* by a yet unknown mechanism, they are not designed with such conversion in mind, as are triptolide prodrugs.) This category may also include prodrugs of triptolide derivatives, e.g. an ester, carbamate or carbonate that undergoes conversion to the derivative *in vivo* via a predicable mechanism such as hydrolysis. Examples include 14-acetyl-5,6-didehydro triptolide and 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide. In one embodiment, the derivative is a synthetic derivative. Derivatives may also include the naturally occurring compounds 16-hydroxytriptolide and tripdiolide (2-hydroxytriptolide).

With regard to structure, a "derivative" of triptolide preferably refers to a compound derived from triptolide via a modification which can include, for example: substitution of a hydrogen atom or hydroxyl group with hydroxyl, lower alkyl or alkenyl, lower acyl, lower alkoxy, lower alkyl amine, lower alkylthio, oxo (=O), or halogen; or conversion of a single bond to a double bond or to an epoxide. In this sense, "lower" preferably refers to C_1 to C_4, *e.g.* "lower alkyl" preferably refers to methyl, ethyl, or linear or branched propyl or butyl. Preferred hydrogen atom substitutions include hydroxyl, methyl, acetyl ($C(O)CH_3$) and fluoro.

Triptolide derivatives and prodrugs useful in the invention are not intended to be limited to the exemplary compounds discussed above. For examples of further derivatives and prodrugs, see the U.S. patents and applications cited above.

Derivatives and prodrugs with "immunosuppressive activity" can be identified via standard *in vitro* and *in vivo* assays. *In vitro* assays include the IL-2 inhibition assay described in co-owned PCT Pubn. No. WO 2003/101951; compounds may also be tested for inhibition of TGF-β, using commercially available kits, such as the TGF-β Emax® ImmunoAssay System provided by Promega Corporation. Preferably, immunosuppressive

activity is such that the target cytokine is inhibited by the triptolide compound at a concentration at most 50 times greater, more preferably at most 10 times greater, and most preferably at most 5 times greater than the concentration of native triptolide that provides the same level of inhibition in the assay.

II. Therapeutic Compositions

Formulations containing triptolide compounds for use in the methods of the invention may take the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as tablets, capsules, powders, sustained-release formulations, solutions, suspensions, emulsions, ointments, lotions, or aerosols, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions typically include a conventional pharmaceutical carrier or excipient and may additionally include other medicinal agents, carriers, or adjuvants.

Preferably, the composition will be about 0.5% to 75% by weight of a compound or compounds of the invention, with the remainder consisting of suitable pharmaceutical excipients. For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. If desired, the composition may also contain minor amounts of non-toxic auxiliary substances such as wetting agents, emulsifying agents, or buffers.

The composition may be administered to a subject orally, transdermally or parenterally, *e.g.*, by intravenous, subcutaneous, intraperitoneal, or intramuscular injection. For use in oral liquid preparation, the composition may be prepared as a solution, suspension, emulsion, or syrup, being supplied either in liquid form or a dried form suitable for hydration in water or normal saline. For parenteral administration, an injectable composition for parenteral administration will typically contain the triptolide derivative in a suitable intravenous solution, such as sterile physiological salt solution.

Liquid compositions can be prepared by dissolving or dispersing the triptolide compound (generally about 0.5% to about 20%) and optional pharmaceutical adjuvants in a pharmaceutically acceptable carrier, such as, for example, aqueous saline, aqueous dextrose, glycerol, or ethanol, to form a solution or suspension.

The compound may also be administered by inhalation, in the form of aerosol particles, either solid or liquid, preferably of respirable size. Such particles are sufficiently small to

pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about 1 to 10 microns in size, and preferably less than about 5 microns in size, are respirable. Liquid compositions for inhalation comprise the active agent dispersed in an aqueous carrier, such as sterile pyrogen free saline solution or sterile pyrogen free water. If desired, the composition may be mixed with a propellant to assist in spraying the composition and forming an aerosol.

Methods for preparing such dosage forms are known or will be apparent to those skilled in the art; for example, see Remington's Pharmaceutical Sciences (19th Ed., Williams & Wilkins, 1995). The composition to be administered will contain a quantity of the selected compound in an effective amount for effecting immunosuppression, particularly cytokine inhibition, in a SARS patient as described herein.

III. Treatment Methods

In accordance with the invention, administration of a triptolide compound is expected to prevent the development of fibrotic sequelae in SARS, as well as non-fibrosis-related lung pathology resulting from cytokine production and the cytokine-induced effects upon lung tissue, aspects of SARS that are more acute and life threatening than fibrosis. Fibrosis, which usually develops in a chronic manner, has been seen after recovery in a small percentage of SARS patients (Drosten *et al.*, 2003).

A range of doses is practical for this treatment. Results from a phase I clinical trial with a triptolide prodrug, triptolide succinate sodium salt (designated PG490-88Na), show that a dose of 0.675 $\mu g/m^2$ administered by i.v. infusion is well tolerated with no drug-related toxicity. This dose calculates to about 20 μg/kg. Treatment in this clinical study is administered at weekly intervals.

For administration to human patients, a reasonable range of doses, for a prodrug that converts to triptolide in human plasma at a rate similar to that of triptolide succinate, is 1 μg/kg to 100 μg/kg. For derivatives which do not require conversion for activity, such as 14-deoxy-14α-fluoro triptolide, a lower dose range will be useful, such as 0.1 to 40 μg/kg, depending upon the activity of the derivative compared to that of triptolide.

It is reasonable to treat SARS patients several times per day by i.v. infusion with the triptolide prodrugs or derivatives, or possibly by continuous infusion, as dictated by their clinical state and response to the treatment. With more frequent, or continuous treatment, the dose on a $\mu g/m^2$ or μg/kg basis would be reduced. While i.v. administration is

preferred in a clinical setting, other modes of administration, such as parenteral or oral, may also be used, with higher dosages typically used for oral administration.

IV. Timing of Treatment

Treatment with the triptolide compounds and/or other immunosuppressive agents, as described in this invention, is initiated when the patient's clinical condition warrants intervention. It is important to follow the level of the SARS-CoV and to delay initiation of this treatment until the level of the SARS-CoV in the lungs has declined significantly from a peak level, so that the treatment does not compromise the immune capacity of the patient to the extent that the continued decline of the viral load is interrupted or compromised. Decline in the viral load indicates that the first phase of the disease has passed, and therapy to intervene in the development of the second phase is appropriate. Testing to determine the level of SARS-CoV is therefore advisable in making a decision on the initiation of treatment. Treatment continues until there is clear evidence of efficacy and the viral load has remained at a very low level. The pathology characteristic of SARS must be well controlled, and the viral load must not have increased again after falling from the peak level.

V. Combination Treatment

In a rapidly progressing, life-threatening disease like SARS, combination or multiple treatment is the norm, particularly when the symptoms of the disease are being treated rather than the initial cause of the disease – the virus. The triptolide compounds may therefore be used in combination with other agents. These additional agents include, but are not limited to, antiviral agents, corticosteroids, additional immunosuppressive agents, *e.g.* as described above, and immune potentiators.

Other compounds with immunosuppressive activity include, for example: azathioprine, brequinar, chlorambucil, 2-chloro deoxyadenosine, cyclosporin, cyclophosphamide, 15-deoxyspergualin, dexamethasone, everolimus, fluorouracil, leflunomide, mercaptopurine, methotrexate, mitomycin, mitoxantrone, mizoribine (bredinin), mycophenolate mofetil, prednisone, prednisolone, sirolimus (rapamycin), thalidomide, tacrolimus (FK506), thioguanine, and thiopurine).

The level of cytokines can also be reduced, and the morbidity and mortality of SARS reduced, by the use of biological agents that have specificity for any of the cytokines

produced in a SARS infection or prevent binding of these cytokines to cytokine receptors on target cells. Cytokine antagonists comprised of soluble receptors, antibodies, or binding proteins for the cytokines, or receptors to the cytokines, produced in a SARS infection may contribute to reduction in the cytokine levels. Cytokines such as TNF-α, IL-1β, IL-6, IL-8, IL-18 and others may be involved in the pathogenesis of SARS, and cytokine antagonists that bind to these or other cytokines or their receptors may prevent their biological effects and thus reduce the morbidity and mortality of the SARS infection. Etanercept™ (a soluble TNF receptor antagonist), Infliximab™ (an anti-TNF antibody) and Anakinra™ (a soluble IL-1 receptor antagonist) are examples of cytokine antagonists, and reagents targeting these and other cytokines/cytokine receptors are in preclinical and clinical development.

More than one of the cytokine antagonists described herein may be used in combination. The cytokine antagonists are specifically targeted at a single cytokine pathway. As it is likely that multiple cytokines are involved in the pathogenesis of SARS, it is possible that targeting a single cytokine may not sufficiently reduce the morbidity and mortality of SARS. Combination treatment with triptolide compounds, immunosuppressive agents, and cytokine antagonists may be used to increase the effectiveness of the treatment.

As in any immunosuppressive therapy, it is advisable to monitor aspects of the immune system, to allow modulation of the treatment if necessary.

In addition, lymphopenia is seen in some SARS patients. It is important to avoid exacerbating or extending the period of lymphopenia by the treatment described herein. Accordingly, the lymphocyte level should be monitored, and the treatment dose should be adjusted if necessary to avoid treatment-related lymphopenia.

VI. Virus Detection

Monitoring of the viral level in the lungs is also advisable when practicing this invention. For practical reasons, and based on evidence provided by the WHO (World Health Organization) laboratory network, upper respiratory specimens are most suitable for virus detection (isolation and RNA detection). Sampling from multiple sites increases detection rate. The polymerase chain reaction (PCR) can be used to detect genetic material of the SARS-CoV in various specimens (blood, stool, respiratory secretions or body tissues).

Antibody tests detect antibodies produced in response to SARS-CoV infection. Different types of antibodies (IgM and IgG) appear and change in level during the course of infection. They can be undetectable at the early stage of infection. IgG usually remains detectable after resolution of the illness. The following test formats are being developed:

- ELISA (Enzyme Linked ImmunoSorbant Assay): a test detecting a mixture of IgM and IgG antibodies in the serum of SARS patients yields positive results reliably at around day 21 after the onset of illness.

- IFA (Immunofluorescence Assay): a test detecting IgM antibodies in serum of SARS patients yields positive results after about day 10 of illness. This test format is also used to test for IgG. This is a reliable test requiring the use of fixed SARS-CoV and an immunofluorescence microscope.

Virus in specimens (such as respiratory secretions, blood or stool) from SARS patients can also be detected by inoculating cell cultures and growing the virus. Once isolated, the virus must be identified as the SARS virus with further tests. Cell culture is a demanding test, but it is currently (with the exception of animal trials) the only means to show the existence of live virus.

VII. Evidence of Efficacy

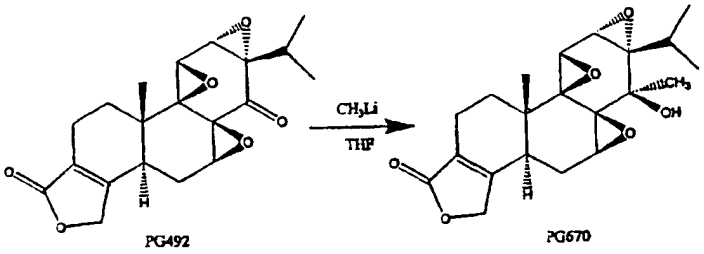
The main evidence for the efficacy of the treatment approach described here is recovery of SARS patients from the disease, and recovery without the serious sequelae associated with SARS infection. The particular cytokine(s) responsible for the lung pathophysiology observed in SARS have not been identified. It is likely that a combination of cytokines is involved. Evaluation of the level of several individual cytokines in bronchoalveolar lavage fluid and or patient plasma may give an indication of the progression of the response to SARS viral infection and of the efficacy of the treatment.

Evidence from other, non-SARS-related strains of human coronaviruses indicates that long-term, protective immunity does not develop, and the same person can be repeatedly infected with the same virus. If protective immunity does not develop with SARS, and reinfection can occur, then repeat treatment with the agents described in this invention may be necessary. The renewed use of this treatment would not be expected to be problematic.

EXAMPLES

Methods of synthesis of various exemplary triptolide derivatives and prodrugs are provided below. All structures were verified by NMR.

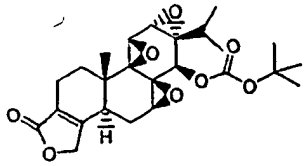
Example 1. Preparation of 14-C-methyltriptolide (PG670)



PG492 PG670

To a solution of triptonide (designated PG492) (60 mg, 0.17 mmol) in THF (5 ml) at –78 °C was added 0.45 ml of methyl lithium (1.4 M solution in ethyl ether, 0.63 mmol, 3.7 eq) under N_2. The solution was stirred at –78 °C for 2 hrs 45 mins and then at room temperature for 2 hrs , at which time the starting material had disappeared on TLC. Acetic acid (1 ml) was slowly added. The solution was then concentrated under vacuum. The crude product was dissolved in dichloromethane (3 ml) and passed through a pad of silica gel, which was then washed with 5% methanol in ethyl acetate (80 ml). After removal of solvent, 78 mg of crude product was obtained. This was dissolved in acetonitrile (0.6 ml) and filtered. The product mixture was separated on HPLC, using a 10x250 mm column of Econosil C18 and a guard column cartridge (7.5x4.6 mm) of Alltima C18, both from Alltech, with mobile phase CH_3CN/H_2O 40/60 with a flow rate of 2.0 ml/min. The sixth peak, having a retention time of 32.13 mins, was collected and concentrated under vacuum. The product had m/z 374 (7.9 mg, yield: 12.6%).

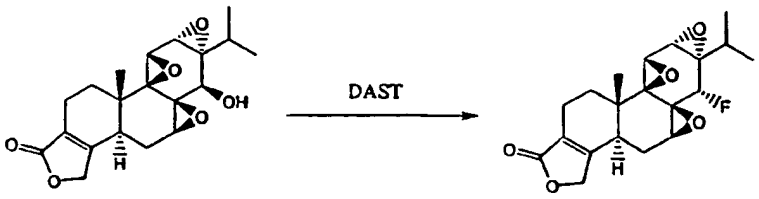
Example 2: Synthesis of Triptolide 14-tert-Butyl Carbonate (PG695)



To a solution of triptolide (108.1 mg, 0.30 mmol, 1.0 eq) and 4-DMAP (367.0 mg, 3.0 mmol, 10.0 eq) in dichloromethane (15 ml) was added with stirring di-tert-butyl dicarbonate (393.0 mg 1.80 mmol, 6.0 eq) at room temperature under nitrogen. After 48 hours of stirring at room temperature, methyl alcohol (1.0 ml) was added. The reaction

mixture was concentrated under vacuum and the crude product was purified via preparative TLC (EtOAc/hexanes/MeOH 1:1:0.1) to give 131.3 mg (95.1%) of product.

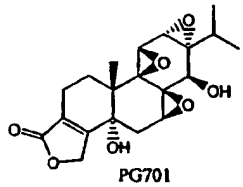
Example 3. Preparation of 14-deoxy-14α-fluoro triptolide



PG490 PG763

To a solution of PG490 (triptolide, 17.3 mg, 0.048 mmol) in dichloromethane (1.0 ml) at 0°C was added (diethylamino)sulfur trifluoride (DAST, 100 µl, 0.763 mmol) under N_2. The reaction mixture was stirred at 0°C for 2 hrs, and saturated $NaHCO_3$ solution (0.8 ml) was then added. The reaction mixture was extracted with 3 x 2 ml of dichloromethane. The combined organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The product (PG763) was obtained in quantitative yield.

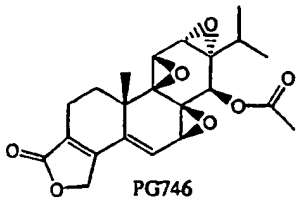
Example 4: Synthesis of 5-α-hydroxytriptolide (PG701)



PG701

To a solution of triptolide (437.6mg, 1.21mmol) in 1,4-dioxane (35mL) was added selenium dioxide (305.1mg, 2.75mmol). The reaction mixture was stirred at 90°C under N_2 for 70 hrs. After cooling to room temperature, the reaction mixture was filtered through Celite and concentrated under vacuum. The crude product was purified via preparative TLC (EtOAc/CH_2Cl2 3:7) to yield the desired product (211.7mg, 46.3%).

Example 5: Synthesis of 14-acetyl-5,6-didehydrotriptolide (PG746)

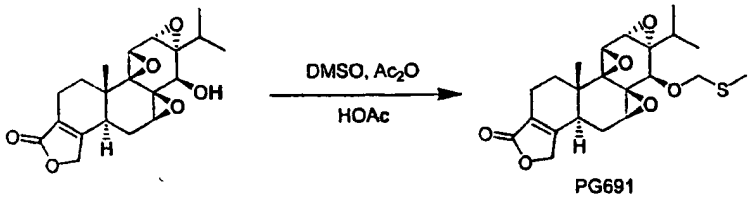


PG746

To a solution of 5-α-hydroxytriptolide (PG701, 98.3mg, 0.261mmol), 4-dimethylaminopyridine (DMAP, 45.2mg) and triethylamine (TEA, 0.50mL) in dichloromethane (5.0mL) was added acetic anhydride (0.247mL, 2.61mmol, 10.0eq) at room temperature under nitrogen. After stirring for 4-5 hrs at room temperature, methanol (1.0mL) was added, and the reaction mixture was concentrated under vacuum. The crude product, 14-acetyl-5-α-hydroxytriptolide, was purified using preparative TLC. To a solution of this material (10.5mg, 0.025mmol), in CH_2Cl_2 (0.50mL) at 0°C was added (diethylamino)sulfur trifluoride (DAST, 4.3 μL, 0.033mmol, 1.3eq). The reaction mixture was stirred at 0°C under N_2 for 40 mins. Saturated aq. $NaHCO_3$ (0.2mL) diluted with 0.3 mL H_2O was added to the reaction mixture at 0°C. The mixture was then extracted with dichloromethane (1.5, 2x2.0mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified using preparative TLC (EtOAc/hexanes/MeOH 40:60:5.0) to yield 4.0 mg product (39.8%).

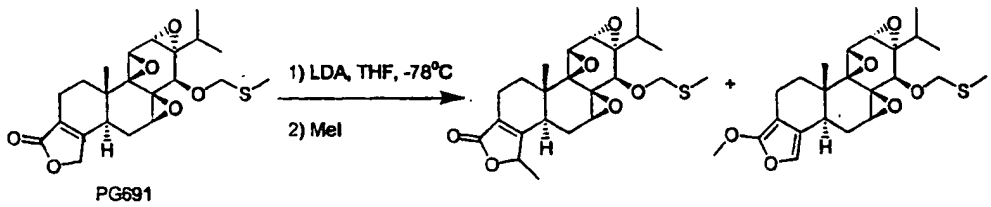
Example 6. Preparation of 19-Methyl Triptolide (PG795)

A. Protection of 14-hydroxyl group



To a solution of triptolide (0.56 g, 1.6 mmol) in DMSO (8.5 mL, 0.12 mol) was added acetic acid (28 mL, .49 mol) and acetic anhydride (5.6 mL, 59 mol), and the solution was stirred at room temperature for five days. The reaction mixture was poured into 200 mL of water and neutralized with solid $NaHCO_3$, added in portions. The mixture was extracted with ethyl acetate (150 mL x 3), and the extract was dried over anhydrous sodium sulfate. and concentrated to give an oil. Silica gel column chromatography purification (3:2 hexanes/EtOAc) gave the intermediate (PG691) (0.45 g, 69%) as a white foam.

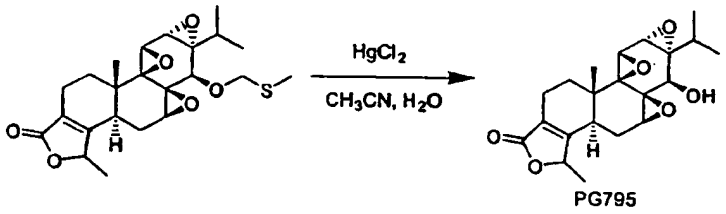
B. Methylation



To a solution of PG691 (0.22g, 0.52 mmol) in anhydrous THF (10 mL) was added a solution of LDA in heptane/THF/ethyl benzene (0.30 mL of 2.0 M solution, 0.60 mmol) dropwise at –78 °C. The solution was stirred at this temperature for 15 min, followed by dropwise addition of CH_3I (50 μL, 0.80 mmol). The mixture was stirred at –78 °C for 2 h, then allowed to come to room temperature overnight.

The reaction mixture was neutralized with 1N HCl and extracted with EtOAc (10 mL x 3). The EtOAc solution was washed with 5% aqueous sodium thiosulfate (10 mL x 2) and dried over anhydrous sodium sulfate. Concentration under reduced pressure gave an oil. Column purification (silica gel, 3:2 hexanes/EtOAc) gave two products, methylthiomethyl protected 19-methyltriptolide (45.9 mg, 20%) and methylthiomethyl protected 18-methoxyfuranotriptolide.

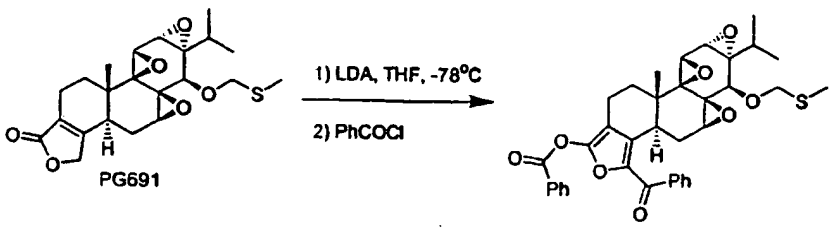
C. Deprotection



To a solution of methylthiomethyl protected 19-methyltriptolide, prepared as described above (45.9 mg, 0.106 mmol), in 1.5 mL acetonitrile/water (4:1) was added mercuric chloride (0.285 g, 1.05 mmol) in one portion. The resulting solution was stirred at room temperature overnight. The white solid which precipitated from the solution was removed by filtration through Celite® and rinsed with ethyl acetate. The EtOAc solution was washed twice with 5% aqueous NH_4OAc. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. Purification by column chromatography (silica gel, 1:1 hexanes/ethyl acetate) gave the product (39.5 mg, 99%).

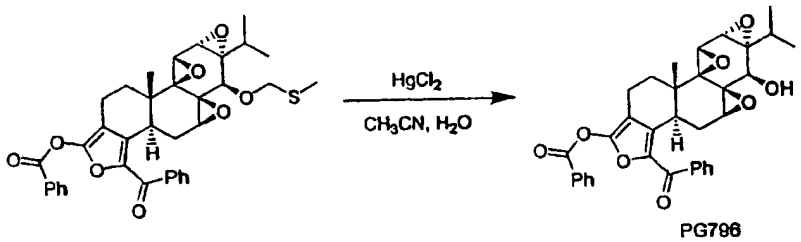
Example 7. Preparation of 18-deoxo-19-dehydro-18-benzoyloxy-19-benzoyl triptolide (PG796)

A. Acylation



To a solution of PG691, prepared as described above (73.1 mg, 0.174 mmol), in anhydrous THF (5 mL) was added a solution of LDA in heptane/THF/ethyl benzene (0.34 mmol) dropwise at –78 °C. The solution was stirred at this temperature for 15 min, followed by the dropwise addition of benzoyl chloride (100 μL, 0.86 mmol). The reaction was stirred at –78 °C for 2 h, then quenched with water and extracted with ethyl acetate (25 mL x 3). The combined organic solution was dried over anhydrous sodium sulfate. Concentration under reduced pressure gave an oil. Column purification (silica gel, 3:2 hexanes/ethyl acetate) gave the 14-protected product (51.2 mg, 47%).

B. Deprotection



PG796

To a solution of the 14-methylthiomethyl protected product, prepared as described above (51.2 mg, 0.0814 mmol), in 1.5 mL acetonitrile/water (4:1) was added mercuric chloride (0.22 g, 0.81 mmol) in one portion. The resulting solution was stirred at room temperature overnight. The white solid which precipitated from the solution was removed by filtration through Celite® and rinsed with ethyl acetate. The EtOAc solution was washed twice with 5% aqueous NH_4OAc. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. Purification by column chromatography provided the pure product (32.8 mg, 71%).

CLAIMS

1. A method of inhibiting cytokine production in a patient infected with Severe Acute Respiratory Syndrome (SARS) coronavirus, comprising administering to such a patient a triptolide compound.

2. The method of claim 1, wherein said compound is triptolide.

3. The method of claim 1, wherein said compound is a triptolide prodrug.

4. The method of claim 1, wherein said compound is a triptolide derivative, or prodrug thereof, having cytokine inhibiting activity.

5. The method of claim 1, wherein said administering is initiated when the level of SARS-CoV in the lungs of the patient has declined from a peak level.

6. The method of claim 5, further comprising administering to said patient an additional therapeutic agent selected from an antiviral agent, an antiinflammatory agent, an additional immunosuppressive agent, and an immune potentiator.

7. The method of claim 3, wherein said triptolide prodrug is a pharmaceutically acceptable triptolide 14-succinate salt.

8. Use of a triptolide compound selected from triptolide, a triptolide prodrug, and a triptolide derivative or prodrug thereof having cytokine inhibiting ability for preparation of a medicament for inhibiting cytokine production in a patient infected with Severe Acute Respiratory Syndrome (SARS) coronavirus.

9. The use of claim 8, wherein said compound is triptolide.

10. The use of claim 8, wherein said compound is a triptolide prodrug.

11. The use of claim 8, wherein said compound is a triptolide derivative, or prodrug thereof, having cytokine inhibiting activity.

12. The use of claim 8, wherein said triptolide prodrug is a pharmaceutically acceptable triptolide 14-succinate salt.